PREVALENCE OF ENTEROPATHOGENIC INFECTIONS IN HIV PATIENTS

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BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled **"PREVALENCE OF ENTEROPATHOGENIC INFECTIONS IN HIV PATIENTS"** submitted by **Dr. S. UMA** to the Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the requirement for the award of M.D degree Branch – XII A (Dermatology, Venereology & Leprosy) is a bonafide research work carried out by her under direct supervision & guidance.

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DECLARATION

I, **Dr. S. UMA** declare that, I carried out this work on, **"PREVALENCE OF ENTEROPATHOGENIC INFECTIONS IN HIV PATIENTS"** at the Department of Sexually Transmitted Diseases, Govt. Rajaji Hospital during the period of June 2007 to August 2008. I also declare that this bonafide work or a part of this work was not submitted by me or any others for any award, degree or diploma to any other University, Board, either in India or abroad.

This is submitted to The Tamilnadu Dr. M. G. R. Medical University, Chennai in partial fulfillment of the rules and regulations for the M.D. Degree examination in Dermatology, Venereology & Leprosy.

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ABBREVIATIONS

WHO	-	World Health Organisation
HIV	-	Human Immuno Deficiency Virus
AIDS	-	Acquired Immuno Deficiency Syndrome
CD	-	Cluster Differentiation
ELISA	-	Enzyme Linked Immuno Sorbent Assay
ART	-	Anti Retroviral Therapy
AFS	-	Acid Fast Staining
HAART	-	Highly Active Anti Retroviral Therapy

INTRODUCTION

HIV / AIDS is becoming a major threat to the human population across the Globe. According to UNAIDS report, globally there were an estimated 33 million people living with HIV in 2007. There were about 2.7 million new HIV infections and about 2 million AIDS related deaths in 2007. The rate of new HIV infections has fallen in several countries, but has increased in developing countries.

Patient infected with HIV suffer from various opportunistic infections, the most commonly encountered in our setting being Tuberculosis, Candidiasis, Pneumocystis jiroveci Pneumonia and diarrhoea due to various pathogens. Intestinal opportunistic infections present commonly as diarrhoea.

Studies indicate that diarrhoea occurs in 30-60% of AIDS patients in developed countries and more than 90% in developing countries.

Chronic diarrhoea is responsible for considerable morbidity and mortality in such patients. Several species of protozoa and other bacterial infections have been associated with acute and chronic diarrhoea in HIV disease.

Only a few studies regarding the prevalence of intestinal opportunistic infections in HIV infected patients are available from South India at present.

I took up this study in our institution, to evaluate the prevalence of such infections in HIV patients in our setup and to ascertain the importance of stool examination for the detection of enteropathogens that aids in treatment, thereby decreasing the morbidity and mortality due to opportunistic gastrointestinal infections.

AIM OF THE STUDY

1. To study the prevalence of enteropathogenic infections in HIV positive patients.
2. To study the correlation between CD4 and the prevalence of intestinal parasites.
3. To study the association between the gastrointestinal symptoms and the enteropathogenic infections in our setting.

REVIEW OF LITERATURE

India has the dubious distinction of having the largest number of people living with Human immuno deficiency virus in the world.[1] with an adult prevalence rate of 0.91% [2].

People with advanced HIV induced immuno suppression are vulnerable to infections called "Opportunistic infections" because they take advantage of the opportunity offered by weakened immune system. Since the beginning of the HIV epidemic, opportunistic infections have been recognized as common complications of HIV infections. [6] [7] [8] Opportunistic infections cause substantial morbidity and hospitalization, necessitate toxic and expensive therapies, and shorten the survival of people with HIV infection [9,10].

The relative frequencies of specific opportunistic diseases may vary in different countries and even in different areas within the same country. [12]. The identification of such pathogens is very important for HIV & AIDS case management.

Diarrhoea is one of the most common presenting complaints in symptomatic HIV infected individuals. The infectious aetiological agents include both opportunistic agents that consistently cause severe,

chronic or frequent gastro intestinal disease and non-opportunistic agents, that usually cause acute, treatable diarrhoeal illness. [3]. It is associated with a 3.3 fold increased risk of disease progression. [3]

Chronic diarrhoea, defined as persistence of diarrhoea beyond four weeks [4] is a common symptom in HIV infected patients in the tropics. The World Health Organisation (WHO) defines diarrhoea wasting syndrome along with a positive HIV serology test to be an AIDS defining illness. [5].

Opportunistic parasitic gut infections cause severe diarrhoea and profoundly compromise the absorptive function of the small intestine, leading to significant mortality.[13]

Protozoan parasites, namely *Cryptosporidium parvum, Isospora belli, Cyclospora cayetanensis, Microsporidia, Entamoeba histolytica / Entamoeba dispar, and Giardia lamblia* account for a significant number of cases of diarrhoea in this population[14]. They are described in detail below. Giardiasis is not more frequent or severe than in HIV negative individuals [15].

Cryptosporidium (spp) :

This small, coccidian parasite was considered a veterinary pathogen but not an important human pathogen until the early 1980's, when it was recognized in patients with AIDS. Subsequently, it has become recognized as a major cause of diarrhoeal illness both in immunocompromised and immunocompetent individuals. [18]

Only two species are commonly seen in humans, *Cryptosporidium hominis*, which has anthroponotic transmission and *Cryptosporidium parvum*, which is seen in humans mainly in outbreak settings, and has a zoonotic transmission cycle. Other species are rarely seen in immunocompetent individuals, but multiple species have been described in HIV infected individuals in developing countries.

Cryptosporidium hominis causes acute watery diarrhoea in immuno competent individuals and protracted life threatening diarrhoea in immuno deficient individuals. This is considered an AIDS defining illness. Recently, following multiple out breaks of diarrhoea, including one affecting over 4,00,000 people following contamination of water supplies, it has also been described as a water borne pathogen, which is difficult to eradicate.[20]

This coccidian has a worldwide geographical distribution with increased incidence in developing countries. Prevalence rates of over 12% are seen in asymptomatic individuals. Transmission can be by faeco-oral route, water borne, due to contact with farm animals and by drinking unpasteurized milk. [31]

The incubation period ranges from 1 to 14 days. Typically acute infection is characterized by watery diarrhoea, crampy epigastric abdominal pain, weight loss, anorexia, malaise and flatulence. [21] [22]

Spectrum varies from asymptomatic shedding to chronic diarrhoea depending on the CD4 count. Median CD4 count of individuals who develop chronic severe diarrhoea is well under 50 cells / mm^3. [21, 22]

All patients with CD4 count > 200 cells / mm^3 are able to clear cryptosporidial enteritis. [23, 36]

A subset of patients with AIDS, cryptosporidiosis develop biliary tract involvement associated with right upper quadrant pain, nausea & vomiting [22, 24]. This is a difficult to treat complication in individuals with exceedingly low CD4 count and long standing chronic cryptosporidial enteritis. [25]

The most common site of cryptosporidia infection is the small intestine, although it is frequently present in the colon and the biliary tract of persons with immunodeficiency. It infects only the epithelial surface of the mucosa and it does not invade the submucous layer or cause ulcerations. [23] [26] [27].

Lab Diagnosis :

The stage seen in faeces is the oocyst which measures 4-6 µm and contains 4 fully developed and infectious sporozoites. 80% are thick walled and 20% thin walled.

Oocysts stain with modified acid fast stain and auramine phenol stains, appearing bright orange red or fluorescing golden yellow under ultraviolet illumination. Other stains used include methanamine silver, Giemsa and periodic acid Schiff stains. Polyclonal and monoclonal antibody conjugates with flourescein isothiocyanate for direct identification of oocysts in stool are also available commercially. Identification of antibodies to *cryptosporidium* measures exposure of the population to this parasite, but is not useful in diagnosis. [28].

A large number of studies on treatment of this infection have not identified any drug that are useful in a majority of cases. Therapy has

included antimotility agents, Paromomycin, Spiramycin and Azithromycin [29].

Recently, Nitazoxanide has been recommended as specific therapy for cryptosporidiosis but appears effective only in immuno competent patients. Anti retroviral treatment resulting in restoration of immune function results in prevention and resolution of disease.[30] Somatostatin analogues especially octreotide are found to be useful in the treatment of Cryptosporidial diarrhoea.

Isospora belli :

First described in 1860 by Virchow, this diarrhoeal disease pathogen is a host specific coccidian protozoan parasite. There is no evidence that the human Isospora species is capable of infecting non human hosts or that animal species affect humans [31].

Infection with this opportunistic parasite is considered an AIDS defining illness if present for over 4 weeks.[5] It has a world wide geographical distribution.

Transmission may be by faeco-oral, water borne or sexual routes. The incubation period ranges from 1-4 days and can be upto 1 week. The clinical features of infections with *Isospora belli* are diarrhoea, malabsorption, weight loss and abdominal pain. [31].

Diagnosis of infection is by examination of stool samples. Oocysts can be demonstrated in wet preparation and stained with modified acid fast stains and direct fluorescent stains. Duodenal aspirates can also be examined. Small bowel biopsies may show developmental stages of the parasite.[31]

Oocysts are the diagnostic forms of the parasite found in humans. They are elliptical in shape and measure 20-33 µm by 10-19 µm.

Each oocyst contains two sporoblasts, each of which contains four sporozoites.

Treatment with trimethoprim and sulphamethoxazole for 10 days have been shown to eradicate infection, although there is a 50% risk of relapse if treatment is not fully completed. Pyrimethamine can also be used. [17].

Cyclospora cayetanensis :

This organism was first described in humans in Papua New Guinea in 1979. It is a coccidian parasite of the genus *Cyclospora.* [31].

This parasite has a world wide distribution. Transmission by contaminated water supplies has been shown to be responsible for out breaks in many settings, and it is likely that this is also transmitted by the faeco-oral route [32].

Ingestion of the mature oocyst results in watery diarrhoea, weight loss, abdominal pain, fatigue, anorexia, bloating, flatulence and fever. The illness may be prolonged, lasting 2-6 weeks. It is chronic and severe if untreated especially in patients with AIDS [31].

The life cycle is incompletely described. The unsporulated oocyst is shed in the faeces and upto 40% of these sporulate in 1-2 weeks. The oocyst is round, 8-9 µm in diameter similar to *cryptosporidia* but larger, when examined under ultraviolet illumination, exhibiting a blue fluorescence. [33].

Diagnosis of infection is by identification of the oocysts in wet preparations and by staining with modified acid fast stains as for cryptosporidium. It does not stain with monoclonal antibodies specific for cryptosporidium. They have also been observed in duodenal aspirates and small bowel biopsies by electron microscopy [31].

Microsporidia :

The first human case was reported in 1959.

Five main species that affect humans are *enterocytozoon, encephalitozoon, microsporium, pleistophora and Nosema.* They are mainly seen in immunocompromised hosts, although some occasionally cause self limiting diarrhoea in travellers. [18]

Ingestion with *microsporidia* may be

i) Latent asymptomatic or chronic mild symptomatic in adults with normal immunity.

ii) Acute, potentially fatal in neonates.

iii) Proliferative disease in the absence of competent host defences.

The route of transmission is not known, although infection by aerosols or ingestion from animals and social partners have been proposed as potential modes.

Laboratory diagnosis of these infections is by demonstration of *microsporidia* in infected tissue or body fluids. Stool specimens can be examined by the modified trichrome stain, chemifluorescent stain or the Giemsa stain on concentrated specimens.

Microsporidia can also be identified in tissue specimens by electron microscopy. Immunoflurescence using polyclonal antibodies has also been done. ELISA, Indirect immuno fluorescence have been shown to be useful for antibody detection.[34].

Albendazole is the most promising agent. The drug is administered in a dosage of 400 mg twice daily orally for 2 to 4 weeks.

Prophylaxis is indicated in any HIV infected patient with CD4 counts less than 200 cells / mm^3 [31]

HIV and other Helminths

The most common pathogenic helminth associated with AIDS is S*trongyloides stercoralis.* Other helminths reported were A*scaris lumbricoides,Trichuris trichura, Ankylostoma duodenale, Schistosoma mansoni , Hymenolepsis nana.*

Strongyloides stercoralis

A number of early case reports documented disseminated strongyloidiasis in HIV infected patients, but later studies seemed to indicate that the risk of disseminated disease was not associated with HIV related immuno deficiency. This has been proposed to be due to the association of declining CD4 counts with lower larval maturation in the gut. This would diminish the risk of autoinfection which in turn is required for hyperinfection [34].

However, possible immune reconstitution disease with *Strongyloides stercoralis* hyperinfection is now being reported in patients started on anti retroviral therapy. Symptoms may have been due to immune responses to preexisting disseminated infections or

because immune recovery facilitated dissemination of stongyloidiasis[35].

Entamoeba histolytica

Entamoeba histolytica in the immuno compromised patients is believed to be usually a commensal belonging to nonpathogenic zymodemes [16].

Intestinal infection is quite common among homosexuals, the incidence is 20-30%. Presence of trophozoites in fresh stools is pathognomonic.

Clinical presentation of the patients is similar to that reported in non HIV patients.

Blastocystis hominis

This pathogen is also frequently identified in human stools, but the role of this anaerobic protozoan as a human pathogen is controversial and as yet unresolved [17].

Ascaris lumbricoides :

Co-infection of HIV with roundworms is particularly important. Adult round worms reside in the small intestine, but larvae migrate through tissues.

Ascaris polarizes the immune response in young adults to Th2, which increases the risk of sexual transmission of HIV.

Ascaris also suppresses IL-2, a Th1 cytokine that can be used for AIDS / HIV because it improves count of CD4 T cells and restores immune function substantially.

Bacterial entero pathogenic Infections in HIV / AIDS

Bacteria may be responsible for secondary infection of the gastro intestinal tract. Infections with enteric pathogens such as *Salmonella, Shigella and Campylobacter* are more common in homosexual men and are often more severe and more apt to relapse in patients with HIV infection. Patients with untreated HIV have approximately a 20-fold increased risk of infection with *Salmonella typhimurium*.

They may present with a variety of non specific symptoms including fever, anorexia, fatigue and malaise of several weeks duration. Diarrhoea is common but may be absent. Diagnosis is made by culture of blood and stool [37].

HIV infected patients also have an increased incidence of *Salmonella typhi* infection in areas of the world where typhoid is a problem. *Shigella spp,* particularly *Shigella flexneri,* can cause severe

intestinal disease in HIV infected individuals and upto 50% of patients will develop bacteremia [34].

Campylobacter infections occur with an increased frequency in patients with HIV infections. While *Campylobacter jejuni* is the strain most frequently isolated. Infections with many other strains have been reported. Patients usually present with crampy abdominal pain, fever and bloody diarrhoea.

Infection may present as proctitis. Stool examination reveals the presence of faecal leucocytes. Systemic infection can occur, with up to 10% of infected patients exhibiting “bacteremia”. Most strains are sensitive to erythromycin.

MATERIALS AND METHODS

Setting :

The study was conducted in both in-patients and out-patients of Govt. Rajaji hospital.

Collaborating Departments :

i) Institute of microbiology, Madurai Medical College.

ii) Anti retroviral therapy centre / Out patients

Design of Study :

Prospective analytical study

Study period :

June 2007 to August 2008, a period of 15 months

Sample size :

100 consecutive HIV positive patients were included irrespective of the symptomatology, CD4 count and ART status.

Ethical Clearance :

Obtained

Consent :

An informed consent was obtained from each patient.

Selection Criteria :

Inclusion criteria :

i) All men and women who were positive for HIV by two rapid tests and ELISA who presented with or without diarrhoea were included in the study.

ii) All patients were above 15 years of age.

Exclusion Criteria :

1. Patients under 15 years of age
2. Endocrine disorders
3. Malignancy
4. Collagen vascular disorders
5. Other organ disorders
6. Patients on steroid therapy
7. Pregnancy
8. Protein losing enteropathy
9. Crohn's disease
10. Irritable bowel syndrome
11. Unconscious / Bedridden
12. Non co-operative and non-willing
13. Patients who were on antidiarrhoeal or on antimotility drugs
14. Patients using liquid paraffin / laxatives
15. Renal disorders
16. Cholestasis

Materials :

Data Collection :

Socio demographic and clinical data were collected.

- CD4 count was done in all the patients
- General investigations were done, and physician's opinion to rule out the other potential causes of gastrointestinal disturbances was obtained for each patient.

Patients were explained about the proposed study and asked to collect stool samples. Stool samples were collected in two containers. One containing glycerol phosphate buffer and other containing formol saline. For suspected cases of cholera, stool samples were sent in Venkatraman Ramakrishnan Medium. Stool samples were subjected to the following methods for evaluation of parasites

1. Wet saline method
2. Wet Iodine method
3. Floatation technique
4. Sedimentation technique
5. Modified acid fast technique
6. Stool culture

Collection of stool :

For stool sample collection essential criteria followed were

1. Fresh stool samples
2. Receptacle was kept clean and without antiseptics
3. Patients were instructed not to mix urine with stool

Samples were taken to the microbiology department, within 30 minutes.

Preparation of Materials :

Two smear preparations, one unstained preparation and another stained with Lugol's Iodine were made.

a) Wet Saline method :

Minute portion of faeces is diluted with normal saline 0.9% and a drop of it is taken on a clean microscopic glass slide. A coverslip No. 1 or No. 0 is then gently put over it to spread out the emulsion. Unstained preparation is specially useful for demonstration of actively motile forms of parasites like *Entamoeba histolytica*.

b) Wet Iodine Mount :

A drop of Lugol's Iodine is added to minute portion of faeces on a clean microscopic glass slide. A cover slip is placed and examined under microscope. This method helps in the visualization of the cysts of protozoa.

c) Floatation technique :

Faecal material was dissolved in a medium of higher density than the eggs and cysts of parasites. The eggs and cysts that float to the top are collected by placing a glass slide on the surface of the meniscus at the top of the tube.

d) Sedimentation techniques :

Faecal material was dissolved in a solution of density below that of eggs. So eggs will be concentrated at the bottom.

e) Modified Acid Fast staining Method :

It was adopted to detect cryptosporidiosis and isosporidiosis. The details are given below.

Preparation of smear :

1) With Diamond pencil lab numbers were written in full. One glass slide was used for each specimen.
2) Using 5mm internal diameter 24 SWG Nichrome wireloop smears were prepared with a portion of thickest part on about 2/3rd of slide
3) Slides were air-dried
4) The slides were transferred to hot plate 80C and were fixed for 10 mts.

Staining : 'Kinyoun' staining

This staining technique was adopted for Acid Fast Staining method

1. The slides were placed on the staining rack with the smear part upper most, the slides not touching each other.
2. The slides were flooded with carbol fuchsin stain. The slides were heated till carbol fuchsin starts steaming. More carbol fuchsin is added to prevent the slides from drying. The slides were allowed to stain for 9 minutes.
3. The slides were washed well with running tap water.
4. Slides were decolorized by covering completely with 1% sulphuric acid for 30 seconds.

5. Slides were washed again gently with running tap water.
6. Slides were counter stained with 1% methylene blue for one minute.
7. Slides were washed as before with water and the slides were dried with filter paper and the smears were read.

Cryptosporidium appeared as round or oval shaped pink coloured oocysts measuring 4-6 µm.

Isospordium appeared as mature or immature oocysts measuring 20-40 µm

Staining for *microsporidia* : Strong Trichrome stain

Faeces fixed in 10% formalin were used for a minimum of one hour for staining microsporidia.

1. A suspension of faeces in 10% formalin (approx. 1 : 1) was prepared. A thin smear was made along a microscope slide using an orange stick. Some thick areas were made at one end of the slide. The slides were air dried and fixed in methanol for 5 minutes.
2. Slides were stained for 10 minutes in strong Trichrome stain which has been pre-heated to 50^0 C, or 1 hour in cold strong Trichrome stain. Rinsed in tap water to remove excess stain.

3. Rinsed briefly in acid alcohol, for 1-2 seconds. Rinsed briefly in 95% ethanol for 1-2 seconds.
4. Slides were dehydrated in 95% ethanol for 5 minutes and in absolute alcohol for 5 minutes.
5. Microsporidia stain strong pink and measure 1.5 to 2.0 micro meter with a clear vacuole and membrane fold.

Stool Culture for Entero pathogenic bacteria :

For enteric pathogens culture was done on Nutrient agar and Mac conkey agar plates. Further identification was done by biochemical reactions.

Analysis :

Data were entered in Microsoft Excel Spreadsheet and were analysed using statistical package.

Conflict of interest : Nil

Financial support : Nil

Limitations of the Study :

1) Infective status of the parasitic infestations were not supported by serological studies
2) Re-evaluation of the negative cases was not attempted

3) Therapeutic outcome of the patients with intestinal parasitosis were not studied.
4) Post ART follow up of the patients receiving ART was not analysed in relation to non ART patients.

The results of similar studies conducted across the Indian subcontinent were as follows :

Kava Mohandas et al. Dept of Parasitology and Dept. of Int. Medicine, PGI Chandigarh. Prevalence of Intestinal parasitic pathogen in HIV positive patients in Northern India. Jpn, J. Infect. 2002. Dis : 55 : 83-84.

Of 120 HIV positive patients 30% were found to harbour an intestinal parasite. *Cryptosporidium parvum* was the most common (10.8%), followed by *Giardia lamblia* (8.31%). *Cyclospora cayetanensis and Blastocystis hominis* each were detected in 3.3% of the patients, while *Isospora belli* and *Enterocytozoon bieneusi* were each detected in 2.5% of the patients. The other parasite observed were *Entamoeba histolytica* in two cases and hook worm ova in one patient.

Gupta et al. Indian Journal of Medical Microbiology(2008) 26(2) : 172-5 (All India Institute of Medical Sciences)

Out of 113 HIV positive patients, enteric parasites were detected in 55.8% with diarrhoea compared to 16.4% in patients without diarrhoea.

Isospora belli was found in 41.1% of chronic diarrhoea, 6.3% in non diarrhoeal cases. *Cryptosporidium* was detected in 20.6% of chronic diarrhoea and 2.5% in non diarrhoeal cases. *Cyclospora cayetanensis* associated diarrhoea was detected in only one case of chronic diarrhoea (2.9%)

The mean CD4 T cell count was lower (180cells/µl) in diarrhoeal patients as compared to non diarrhoeal patients(261.3cells/µl). Coccidian parasites were seen at a mean CD4 T cell count of 186.3 cells / sec. This study concluded that *Isospora belli* was the predominant parasite followed by cryptosporidium and both were strongly associated with diarrhoea among HIV patients.

Satheesh Kumar, S. Ananthan et al. Dept. of Microbiology, Dr. ALM PG Institute of Basic Medical Sciences, Chennai, India.

During may 2000 to Jan 2001, 152 stool samples from (43 with acute diarrhoea, 59 with chronic diarrhoea and 50 without diarrhoea)

HIV seropositive individuals were examined for enteric coccidian and other intestinal parasites by microscopy and special staining methods.

A total of 52 enteric parasites, 15 from patients with acute diarrhoea and 24 from patients with chronic diarrhoea, 7 from patients infected with HIV without diarrhoea and 6 from normal individuals without diarrhoea.

Isospora belli was detected in 13.7% with acute and chronic diarrhoea. The association with diarrhoea among HIV positive individuals was significant. *Cryptosporidium* was detected in 7 patients each with acute and chronic diarrhoea. It was also detected in 4 patients with HIV infection without diarrhoea. Hence its association with diarrhoea among HIV patients was found to be not significant in the present study. *Cyclospora and microsporidia* each were detected in only one HIV positive patient with chronic diarrhoea.

The study revealed that the coccidian parasites are one of the etiological agents of diarrhoea especially of chronic diarrhoea among HIV positive patients.

Isospora belli was found to be a frequent enteric parasite associated with diarrhoea among HIV positive patients in Chennai.

1. **Nilanjan Chakraborty, Anirban Mukherjee, et al, ICMR, Virus unit, Kolkata, ID & BG HDS petal, Kolkata, India. Jpn. J. Infect. Dis, 61, 49-53; 2008.** In this study of 125 patients, Enteropathogenic vibrio (47%), cryptosporidial diarrhoea (43%). Escherichia coli infection (42%).

Results of similar studies in Other countries :

Mora CA, Altieri R, Davario M, Lasala M.B. Division of infectious disease, J.M. San martin Hospital school of medicine, Buenos Aires Argentina. 11th International AIDS conference, Int. Conf. AIDS, 1996. July 7-12 : 11: 296 Abstract No 4624.

According to the study out of 376 HIV positive patients who were followed from 1-1-1987 to 12-3-1993, parasites were found in 171 patients of which *Entamoeba histolytica* were found in 48 patients (12.77%). *Escherichia coli* were found in 46 patients (12.23%), *Giardia* were found in 39 patients (10.37%). *Dientamoeba Fragilis* in 23 patients (6%), *cryptosporidiosis* were found in 11 patients (3%) and *Isospora belli* in 4 patients (1%). There is significant correlation between cryptosporidiosis and diarrhoea when CD4 count is below 200 cells / mm3.

Alijandro Carabello, Indhira Ozozco, Bol.Chil Parasitol Intestinal parasitic infection in HIV positive individuals in south eastern Venezuela.

According to this study cryptosporidiosis were found in 22.8%, *Ascaris lumbricoides* in 14.2%, Hookworm in 8.6% and *Trichuris trichuria* in 8.6%. *Isospora belli* in 2.9% According to them 56.5% cryptosporidiosis is found and CD4 count < 200 cells/mm^3 and 32% between 200-500 cells/mm^3 and 12% when CD4 count above 500 cells / mm^3 *Entamoeba histolytica* were found in 28.9% when CD4 < 200 cells/mm^3 and 17.4% when CD4 count between 200-500 cells/mm^3 and 53.7% when CD4 count above 500 cells/mm^3.

AR Meamar et al, Iranian J. Parasitol : vol. 2, No.1 2007, pp 1-6. Dept of Parasitology and Mycology, Faculty of Medicine, Iran University of Medical Sciences and Health services, Tehran, Iran.

A total of 781 HIV + / AIDS patients were submitted to copro parasitological examination from 2003 to 2005. 191 individuals were at AIDS stage. The prevalence of intestinal parasites was 11.4%.

The prevalence of infection for each helminith and pathogenic protozoan were as follows.

Blastocystis hominis	-	6.1%
Giardia lamblia	-	4.2%
Cryptosporidium Spp	-	0.9 %
Isospora belli	-	0.26 %
Strongyloides stercoralis	-	0.26%
Hymenolepsis nana	-	0.13 %
Rhabditis axei	-	0.13%

All cases of *Cryptosporidium spp and Isospora belli* with clinical picture of severe diarrhoea were exclusively found in those HIV positive patients who were at AIDS stage.

In all patients with *Cryptosporidium Spp,* the mean number of CD4 lymphocyte was 50.1 + 8.8/mm^3, whereas in 2 patients infected with *Isospora,* this number was 137 + 23.8 /mm^3

R E S U L T S

Symptomatology & Sex Distribution :

Table – 1

	With Gastro intestinal symptoms	Without Gastro intestinal symptoms	Total
Males	36	18	54
Females	25	21	46
Total	61	39	100

Out of 100 patients, 54 were males and 46 were females

Out of 54 males 66 %were symptomatic and out of 46 females 54% were symptomatic.

The symptoms included diarrhoea, vomiting, abdominal pain, flatulence, dyspepsia etc.

Marital Status :

Among the 100 patients, 95 patients were married. Only 5 patients were unmarried. Spouses of 62 patients were also HIV positive by ELISA and were under follow up. 34.7% of the couples were discordant in our study.

Sexual Behaviour

96% of men had history of promiscuous sexual behaviour and 4% of men and all women denied history of promiscuity.

Among men, 7.8% had history of homosexual behaviour. Anogenital sex was the mode adopted by these homosexuals.

Distribution of cases in relation to age

Out of 100 HIV positive patients, 72% were below the age group of 40. The age range varied from 25-55 years. The mean age was 40. There was no significant difference among gender with reference to the age. The results are tabulated below.

Table - 2

Age	Male	%	Female	%	Total	%
< 30	6	11.1	12	26.08	18	18
31-40	27	50	26	56.52	54	54
41-50	20	37	6	13.04	25	25
> 50	1	1.9	2	4.36	3	3
Total	54	100	46	100	100	100

Occupation :

Various occupational categories were noted. Most of the males were drivers and agricultural workers. Others were load men, skilled workers and others. Most of the women were home makers.

Socio-economic Status :

95% of our patients belonged to the low socio economic status. The remaining were of moderate socio-economic status.

Educational Status

The educational status was classified into those who completed their primary school education, high school, higher secondary, graduates, technical education and illiterates.

The following were the observation.

Table - 3

Occupation	**Males**	**Females**
Illiterates	10	10
Primary school	12	8
High school	14	14
Higher secondary	10	13
Graduates	5	1
Technical	3	-
Total	54	46

Domicile Pattern :

Table - 4

	Male	**Female**	**Total**
Rural	32	24	56
Urban	22	22	44
Total	54	46	100

Out of 100 patients, 44 patients were from urban areas in around Madurai and 56 patients were from rural side mostly from villages around Madurai.

57.2% of males and 42.8% of females were from rural areas and 42.8% of males and 57.2%of females were from urban areas.

Water sources :

All patients were dependent on protected water supplied by the local bodies. The knowledge about using boiled water was very poor in illiterate patients when compared with the literate.

Hand washing :

Hand washing habits and other hygiene practices were far from satisfactory among our patients, especially among the illiterates.

Table - 5

Prevalence of other opportunistic Infections in relation to CD4 count

	CD4 > 500	CD4 201-499	CD4 < 200
Oral candidiasis	1	4	56
Tuberculosis (Pulmonary and extra pulmonary)	-	3	18
Pneumocystis jiroveci pneumonia	-	1	8
Oral hairy leukoplakia	-	1	28

Oral candidiasis was the predominant opportunistic infection seen in our patients.

Staging of the Disease :

The staging was done based on the WHO staging for the year 2007 and are tabulated as follows.

Table - 6

Stage	Male	%	Female	%	Total
I	10	18.5	11	23.9	21
II	-		-		-
III	27	50	20	43.4	47
IV	17	31.5	15	32.6	32
Total	54		46		100

79% of our patients belonged to Stage III and IV.

Prevalence of Other STDs among the study group

Table – 7

	Male	Female	Total
Genital herpes	6	6	12
Anogenital warts	2	3	5
Candidiasis	2	5	7
Latent syphilis	-	3	3
Bacterial vaginosis	-	2	2
Pelvic inflammatory disease	-	3	3

Genital herpes was the most common STD in both males and females.

PREVALENCE OF ENTERO PATHOGENS FROM OUR STUDY

Prevalence of Enteric pathogens from our study

Sole isolates :

Cryptosporidium - 33

Entamoeba histolytica - 3

Escherichia coli - 1

Isospora belli - 3

Ankylostoma duodenale - 1

Microsporidia - 1

Mixed infections - 23

Cryptosporidia + Entamoeba histolytica - 8

Cryptosporidia + Escherichia coli - 9

Cryptosporidia + Ankylostoma duodenale - 1

Cryptosporidia + Klebsiella - 1

Cryptosporidia+ Enteamoeba histolytica+Klebsiella –1

Cryptosporidia + Escherichia coli + Ankylostoma duodenale - 1

Giardia lamblia + Entamoeba histolytica - 1

Escherichia coli + Escherichia histolytica - 1

Percentage prevalence of enteric pathogens

% of patients harbouring enteric pathogens - 65%

Sole isolates :

Cryptosporidium - 50.76%

Isospora belli - *4.62%*

Microsporidia - *1.54%*

Entamoemba histolytica- *4.62%*

Ankylostoma duodenale- *1.54%*

Escherichia coli - *1.54 %*

Mixed infections - 35.38%

Table – 8

Enteric pathogens in relation to symptoms

	With gastrointestinal symptoms	**Without gastrointestinal symptoms**	**Total %**
Cryptosporidium	27 (41.53%)	6 (9.23%)	50.76 %
Isospora	2 (3.08%)	1 (1.54%)	4.62%
Microsporidia	1 (1.54%)	-	1.54%
Entamoeba histolytica	1 (1.54%)	2(3.08%)	4.62%
Ankylostoma duodenale	1 (1.54%)	-	1.54%
Escherichia Coli	1 (1.54%)	-	1.54%
Mixed Infections	19 (29.23%)	4 (6.15%)	35.38%
Total	52 (80%)	13 (20%)	100%

Table – 9

Pathogens in relation to CD4 Count

	CD4 < 200		CD4 201-499		CD4 > 500	
	Sympto matic	**Asympt o matic**	**Sympto matic**	**Asympt o matic**	**Sympto matic**	**Asympto matic**
Cryptosporidium	25 (38.45%)	1 (1.54%)	1 (1.54%)	5 (7.69%)	-	1 (1.54%)
Isospora belli	2 (3.08%)			1 (1.54%)	-	-
Microsporidia	1 (1.54%)					
Entamoeba histolytica	1 (1.54%)	1 (1.54%)	-	-	-	1 (1.54%)
Ankylostoma duodenale	1 (1.54%)	-	-	-	-	-
Escherichia coli	1 (1.54%)	-	-	-	-	-
Mixed infections	17 (26.14%)	1 (1.54%)	2 (3.08%)	-	-	3 (4.62%)

DISCUSSION

Enteric pathogens are among the most common opportunistic infections and are a major cause of morbidity and mortality in HIV positive individuals world wide.

The prevalence of enteric pathogens shows wide geographic variations and their isolation and treatment carries importance while treating HIV infected patients especially in advanced stage of immuno suppression.

A clear knowledge of the prevalence of various enteric pathogens in a particular area would be of immense help in choosing empirical antimicrobial regimens in resource poor settings.

In the present study enteric pathogens were recovered from 65% of patients. Study conducted by Kava Mohandass et al (14) at PGI Chandigarh whose sample size closely matched our study, showed only 30% prevalence of enteric pathogens, when compared with an alarmingly high prevalence in our study (65%). The CD4 count and prevalence of pathogens in relation to CD4 count or stage of the disease has not been mentioned in their study. The probable explanation that could be offered is that majority of our patients (79%)

were in stage III & IV of the disease, which could account for the increased prevalence of enteric pathogens in our patients.

Variations in socio economic status, personal hygiene and quality of water supply may also have a role in the disparity.

The study conducted by Gupta et al[39] from All India Institute of Medical Sciences showed a prevalence of intestinal parasites in about 28.3% of 113 patients with HIV. CD4 count was done only for 48 patients due to financial constraints. The prevalence in this study again did not match our study probably due to same reasons mentioned earlier.

The study conducted by Kumar S et al[43] at Chennai in 152 HIV positive patients showed a prevalence of 34.21% of enteric parasites, which is also very low compared to our studies, despite geographic proximity to our centre. This study also does not mention the CD4 count of the patients included.

Studies conducted in Iran by AR Meamar et al[40] showed a prevalence of only 11.4% and studies conducted by Morra et al[41] from Argentina showed a prevalence of 45.4% when compared with our study. Variations in the standard of living, hygiene practices and

availability of treatment facilities would account for the gross variation between different countries.

80% of our patients, who harboured an enteric parasite had gastro intestinal symptoms like diarrhoea, vomiting, nausea, belching, flatulence, colicky abdominal pain. Diarrhoea was the predominant symptoms seen in all patients. A similar finding was also recorded in the other studies.

Kava Mohandass et al[14] also showed a similar picture where 75% of patients with an enteric pathogen had diarrhoea.

Gupta et al[39] showed that 55.8% of patients with enteric pathogens were symptomatic, the percentage was comparatively lower than our study.

Kumar et al[43] showed 72% of patients to be symptomatic which also closely correlated with our study.

Studies from abroad did not mention about the symptomatology, or their prevalence with relation to CD4 count.

The most prevalent pathogen isolated in our study was *cryptosporidium* species. Out of 100 patients screened *cryptosporidium* was the sole pathogen isolated in 33 patients and in 21 patients it was seen as a mixed infection. The overall prevalence as a sole isolate was

50.76%. This is by far the highest prevalence reported so far in studies conducted across the world.

The study conducted by Nilanjan et al[42] showed a prevalence of 43% of cryptosporidial diarrhoea in a sample size of 125. It was the most common entero pathogen isolated as in our study, and the percentage prevalence also closely matched our study.

Kava Mohandas et al[14] reported cryptosporidium as the commonest pathogen isolated with a prevalence of 10.8% which was very less when compared with our study, even though both the studies showed cryptosporidium as the predominant pathogen.

Gupta et al[39] reported *Isospora belli* to be the predominant pathogen with a prevalence of 41.1% with chronic diarrhoea and 6.3% in non diarrhoeal cases. *Cryptosporidium* was detected in 20.6% of chronic diarrhoea and 25% of non diarrhoeal cases.

Kumar et al[43] reported *isospora* to be the most prevalent pathogen (13.7%). *Cryptosporidium* was present in 7% patients. Though closely related in sample size and geographic area there is a gross difference between the species isolated and the percentage of prevalence. This is intriguing, and simultaneous, larger, multicentric studies are necessary to get a clearer picture.

AR Meamer et al[40] Iran showed a very low prevalence of *cryptosporidia* of about 0.9%. Mora et al[41] Buenos Airs, Argentina showed a prevalence of *cryptosporidia* in 3% of patients. In both these studies cryptosporidium was not the predominant pathogen and the prevalence was very low.

Alijandro carabellow et al[40] showed a prevalence of *cryptosporidium* to be 22.8% (South Eastern Venezuela) *cryptosporidium* was the predominant pathogen isolated, though in a lower percentage prevalence.

The type of coccidian parasite isolated differed in different geographic regions, majority of the studies showed *cyrptosporidia* to be the most common pathogen isolated, as in our study. However, the percentage varied. Our study showed the highest prevalence.

Though the isolation of *cryptosporidia* was seen in 33 of our patients (50.76%), *Isospora* was found only in 3 of our patients (4.62%), which was comparable with studies by Kava Mohandass et al[14] (2.5%) but studies by Anandan et al[43] revealed higher percentage of (13.7%) of *isospora* and it was the predominant pathogen isolated in Chennai which was discordant with our study.

Gupta et al[39] showed a 16.8% of *isospora* and again it was the predominant pathogen isolated in their study which was not correlating with our study.

AR Meamar et al[40] reported 0.26% of isospora. The probable explanation that could be offered for this disparity may be as follows. An over whelming 79% of our study population were on cotrimoxazole prophylaxis, started fairly early in the course of the disease, as per the WHO guidelines, irrespective of their CD4 counts, which could have offered protection against this coccidian parasite. The percentage of cotrimoxazole prophylaxis in the other studies was not available for comparison.

Microsporidia was isolated in only one patient (1.54%). Study by Kumar et al[43] showed a prevalence of 0.65% in their study. The prevalence closely correlated with our study, whereas other studies did not isolate *microsporidia* at all.

Other protozoans isolated in our study was *Entamoeba histolytica* (4.62%) *Ankylostoma duodenale* (4.61%). *Giardia lamblia* was seen in only one sample along with mixed isolates.

Kava Mohandas et al[14] showed a higher prevalence of Giardia lamblia (8.3%) and a lower prevalence of Entamoeba histolytica (1.6%) when compared with our study.

Kumar et al[43] did not isolate any of these protozoans in their study.

Gupta et al[39] showed a prevalence of *Giardia lamblia* 2.6% and *Entamoeba histolytica* 0.88%. They isolated *strongyloides stercocaralis* in one patient which was not found in our study.

AR Meamear et al[40] showed a prevalence of *Giardia lamblia* in 4.2% which was higher when compared with our study.

Strogyloides stercoralis was isolated in 0.26% and *Hymenolepsis nana* in 0.13% which were not isolated in our study.

Mora et al[40] showed *Entamoeba histolytica* in 12.77% which was lower and *Giardia* in 10.37% which was higher than our prevalence. Their study also isolated *Dietamoeba fragilis* in 6% of patients which was not isolated in our patients.

E. coli was the most common bacterium isolated with prevalence of 1.54% as the sole isolate. *Klebsiella* was isolated in 2 patients as a mixed isolate.

Nilanjan chakraborty et al[42] reported enteropathogenic vibrio (47%) as the most common bacterium isolated. The probable reason for isolating vibrios as the predominant pathogen was that the study was conducted in Kolkata which is known to be endemic for cholera. *E.coli* was reported in 42% which was very high when compared to our study. The other Indian studies have not made a mention about the bacterial isolates in their study. Studies abroad by Mora et al[41] showed a prevalence of *E.coli* in 12.33% which closely correlated with our study.

Mixed infections were isolated in 23 patients (35.38%). *Cryptosporidium* was the predominant pathogen isolated in the mixed infections.

Out of 23 patients 18 (78.2%) had CD4 count < 200. 82.6% of patients with mixed infections were symptomatic. These features highlight the importance of immune system in clearing the enteric pathogens and advanced immuno suppression provides a favourable environment for mixed enteric pathogens.

None of the studies mentioned across the country and abroad did mention about mixed infections.

Majority of enteric pathogens were isolated in patients with CD4 count < 200 / mm^3 . The statistics is shown in the table and most of the patients with low CD4 count were symptomatic.

Gupta et al[39] showed a similar finding as in our study. CD4 T cell count was lower (180 cells/mm^3) in diarrhoeal HIV patients as compared to non diarrhoeal patients(261.3 cells / mm^3). Coccidian parasites were seen at a mean CD4 + T cell count of 186.3 cell / mm^3.

SUMMARY

Entero pathogenic infections cause significant morbidity & Mortality in HIV patients especially in advanced states of immuno suppression.

Socio economic conditions, educational status, unhygienic practices contribute for intestinal parasitosis.

There are wide geographic variations in the prevalence of entero pathogenic infections due to variations in the standards of living, hygiene practices, availability of safe drinking water and literacy rate.

Since large number of our patients belonged to low poverty line, an attempt was made to study the prevalence of enteric pathogens in our population.

A total of 100 consecutive HIV positive patients (54 males ; 46 females) were selected. After thorough clinical examination and investigations to rule out other systemic illnesses, their stools were examined for the gastrointestinal pathogens.

Enteric pathogens were isolated in 65% of our patients.

Cryptosporidium was the most common pathogen isolated which was found in 50.76% of our patients.

The other pathogens isolated were *Isospora belli* (4.62%), *Microsporidia (*1.54%) *Entamoeba histolytica* (4.62%), *Ankylostoma duodenale* (1.54%), *Escherichia coli* (1.54%), Mixed infections in 35.38%.

The prevalence of infections was high in patients with CD4 count < 200 / mm^3 . Most of the patients with a lower CD4 count were symptomatic.

The isolation of *Isospora belli* was very less. It may be explained by the *cotrimoxazole prophylaxis* that is offered to our patients. There are wide geographic variations among the prevalence of enteric pathogens even in areas which are in close proximity to us, in city like Chennai.

Therefore large multicentric studies need to be conducted in wide geographic areas to assess the prevalence of such parasites, so that appropriate therapeutic strategies can be planned for the treatment of such patients.

Since personal hygiene and good standards of living are essential to prevent acquiring these parasites, proper health education regarding hand washing habits, drinking boiled water needs to be emphasized to all the patients. The availability of resources for treatment especially

HAART is mandatory as boosting up the immunity leads to spontaneous clearance of most of the enteric pathogens especially the coccidian parasites.

CONCLUSION

The prevalence of enteropathogenic infections in 100 conseutive HIV positive individuals were studied and the following conclusions were arrived at.

1. Enteric pathogens were isolated in 65% of our study population, and the coccidian parasites were the most common gastro intestinal pathogens isolated.
2. *Cryptosporidium* is the most common enteropathogen isolated.
3. *Isospora* was isolated only in three of our patients.
4. Escherichia coli was the most common bacterial isolate.
5. *Cotrimoxazole prophylaxis* appears to offer a significant protection against *isospora* infection.
6. The prevalence of enteropathogens varies significantly between different centres, despite geographic proximity.
7. 80% of patients who harboured enteropathogens were symptomatic

8. Mixed infections were commonly seen in profoundly immuno suppressed patients. This signifies that advanced immuno suppression paves way for mixed infections.
9. Patients with advanced illness were more symptomatic than those in the early stages.
10. Enteric pathogens were isolated predominantly when the CD4 count was < 200 cells / mm^3

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PROFORMA

ENTEROPATHOGENS IN HIV

1. S. No. :
2. MVD / FVD No. :
3. Name :
4. Age / Sex :
5. Occupation :
6. Education :
7. Socio-economic status :
8. Marital status : Married / Unmarried
   Divorced / Separated
   Widow / Widower
9. High risk behaviour :
   a) Age at 1st intercourse :
   b) Promiscuous / Non promiscuous:
      Premarital / extra marital
   c) No.of partners :
   d) Protected / Unprotected :
   e) Hetero sexual : Anogenital / Genito genital /Orogenital
   f) Homosexual : Anogenital / Orogenital
      Active / Passive / both
10. Others : Alcoholism
    IVDA
    Substance abuse
    Blood transfusion
    H/o Surgery

11. Date of diagnosis as HIV positive :

    Age at Diagnosis :

    Purpose for which HIV testing was done :

12. Blood VDRL :

13. Other STD's :

14. CD4 count :

15. WHO staging :

16. Presenting complaints :

17. Positive clinical findings:

18. Motion Smear : Wet Mount :

    Modified AFB staining :

    Motion culture :

19. USG :

20. Other Investigations :

21. Other opportunisitic Infections :

22. Treatment given :

    PCP prophylaxis : Yes / No

    ART :

    Others :

23. Results (outcome) :

MASTER CHART ABBREVIATIONS

23/7/07.

K. Dis.No.10275 /E4/1/2007.

Govt. Rajaji Hospital,
Madurai – 625 020. Dt.04.07.2007.

Sub: Establishment – Govt. Rajaji Hospital, Madurai – Ethical Committee
Projects approved by the Committee – Intimation – Sent – Reg.

The Ethical Committee of the Govt. Rajaji Hospital, Madurai was held at 12.30 pm. on 04.07.2007 at the Dean's Chamber, Govt. Rajaji Hospital, Madurai, and the following Projects are approved unanimously by the Committee Members.

S.No.	Name of the Student	Name of the Project approved
01)	Dr.S.Uma,MD PG in DVL	Entro Pathogens in HIV Infection.

Please note that the investigator should adhere the following:-

42) She/He should get a detailed informed consent from the patients/participants and maintain confidentially.
43) She/He should carry out the work without detrimental to regular activities as well as without extra expenditure to the Institution or Government.
44) She/He should inform the Institution Ethical Committee in case of any change of study procedure site and investigation or guide
45) She/He should not deviate for the area of the work for which applied for Ethical clearance.
46) She/He should inform the IEC immediately, in case of any adverse events or serious adverse reactions.
47) She/He should abide to the rules and regulations of the Institution.
48) She/He should complete the work within the specific period and apply for, if any extension of time is required, She should apply for permission again and do the work.
49) She/He should submit the summary of the work to the Ethical Committee on completion of the work.
50) She/He should not claim any funds from the Institution while doing the work or on completion.
51) She/He should understand that the members of IEC have the right to monitor the work with prior intimation.

Uma Swaminathan 5/7/07.

Dean/Chairman,
Ethical Committee, Govt. Rajaji Hospital, Madurai.

To
The above PG students through the Prof. & HOD of STD, Govt. Rajaji Hospital, Madurai.